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BIOCHEMICAL AND GENETICAL STUDIES

ON INH METABOLISM

RESPONSIBLE INVESTIGATOR

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Biochemical and Genetical Studies on INH Metabolism

Semi-Annual Report

30 November 1964

Shigeichi Sunahara

Tokyo National Chest Hospital

CONTENTS

PART I

Studies on Isoniazid Inactivation in Monkies

INTRODUCTION

It was established by us beyond question that the inactivation of isoniazid in humans was a genetic trait and there was not only individual but also racial difference. The frequency distribution curve of the biologically active isoniazid serum levels 6 hours after the oral administration of 4 mg/kg of body weight of isoniazid is trimodal, corresponding to the three phenotypes of isoniazid metabolism--rapid, intermediate and slow inactivators. On the other hand, we observed in vitro that there was very remarkable species and drug differences in the acetylation of isoniazid, sulfonamide and PABA by the liver homogenate of various kinds of animals, and the most active site of acetylation was the liver.

The purpose of the present research is to examine the correlation between the blood concentration and the capacity of tissue to acetylate isoniazid using Cynomoldus monkies as animals of experiment.

METHODS & MATERIALS

The blood level of the biologically active isoniazid was measured by the vertical diffusion method, and the capacity of acetylation of the tissue homogenate by Short's method as indicated in our previous reports. The Cynomoldus monkies (Macaca irus) imported from Malaya, Cambodia and Viet Nam were used in the following experiments.

RESULTS

Fig. 1, 2 & 3 indicate the relationship between 4 and 6 hour levels of the biologically active isoniazid plasma levels after the test doses of 4 mg/kg, 8 mg/kg or 16 mg/kg of isoniazid. Fig. 4 & 5 demonstrate the frequency distribution curve of the values. If we take the concentration 4 hours after 4 mg/kg of INH or 6 hours after 8 mg/kg of INH, we have bimodal curves, but it seems difficult to establish a trimodality as in the case of the humans. There is no marked difference in the inactivation of isoniazid among the monkies imported from three different countries as indicated in Fig. 4. In Fig. 6 is shown the acetylating capacity of isoniazid of the liver, kidney and spleen of the monkies. Also in the case of monkies, the liver is the most active organ concerning isoniazid inactivation. The kidney and spleen show sometimes the activity to a certain degree. It remains obscure whether there is any difference in acetylating capacity among the monkies from different districts also in the homogenate experiments or not. Any close correlation was not established between the rate of acetylation in the liver and the blood level 6 hours after the dose of 4 mg/kg of body weight of isoniazid as shown in Fig. 7.

COMMENT & SUMMARY

We have not been successful to establish a trimodal distribution curve of the biologically active isoniazid levels in monkies up to now. The curve was bimodal as far as the test dose and the time of blood collection in the present study were concerned.

Also in the case of monkies, the liver was the most active site of acetylation but the kidney and spleen had the capacity to a certain extent. There was little correlation between acetylating activity of the liver and 6 hour blood level after 4 mg/kg of INH.

Further study on relationship between the rate of acetylation and blood levels other than 6 hours after the dose of 6 mg/kg of isoniazid is now in progress.

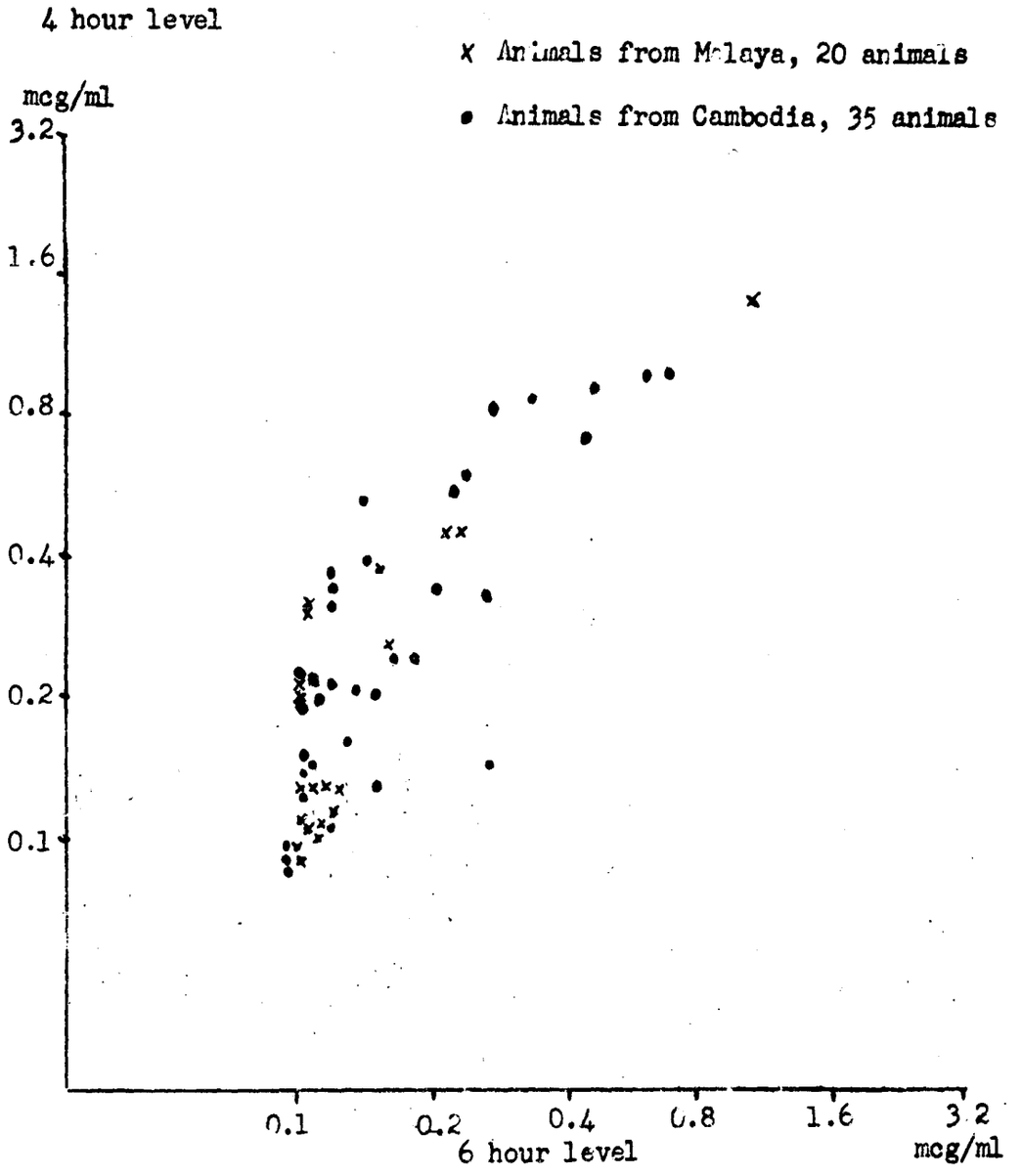


Fig. 1 Relationship between Blood Levels 4 and 6 hours after Administration of 4 mg/kg of INH to Monkies

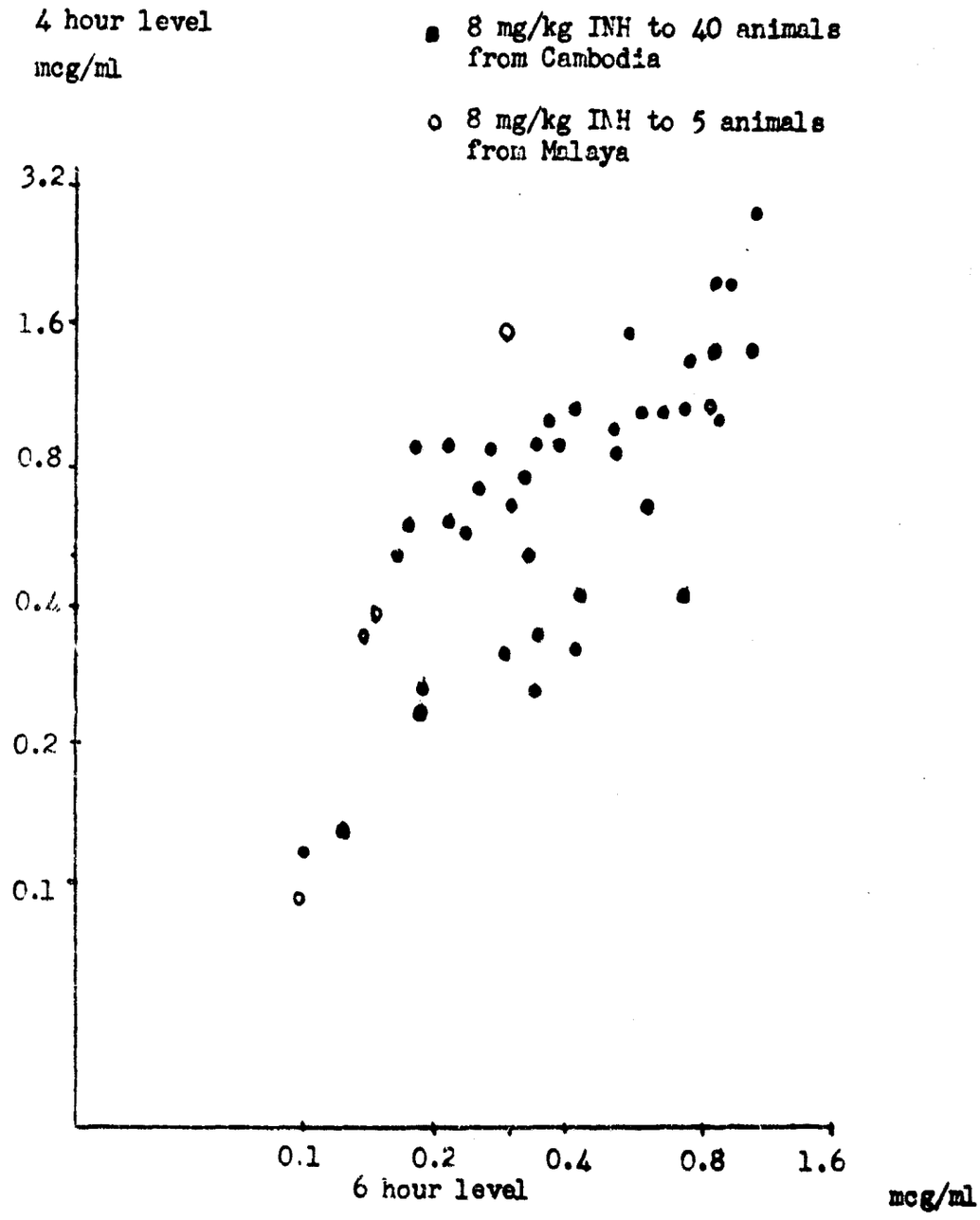


Fig. 2 Relationship between Blood Levels 4 and 6 hours after Administration of 8 mg/kg of INH to Monkies

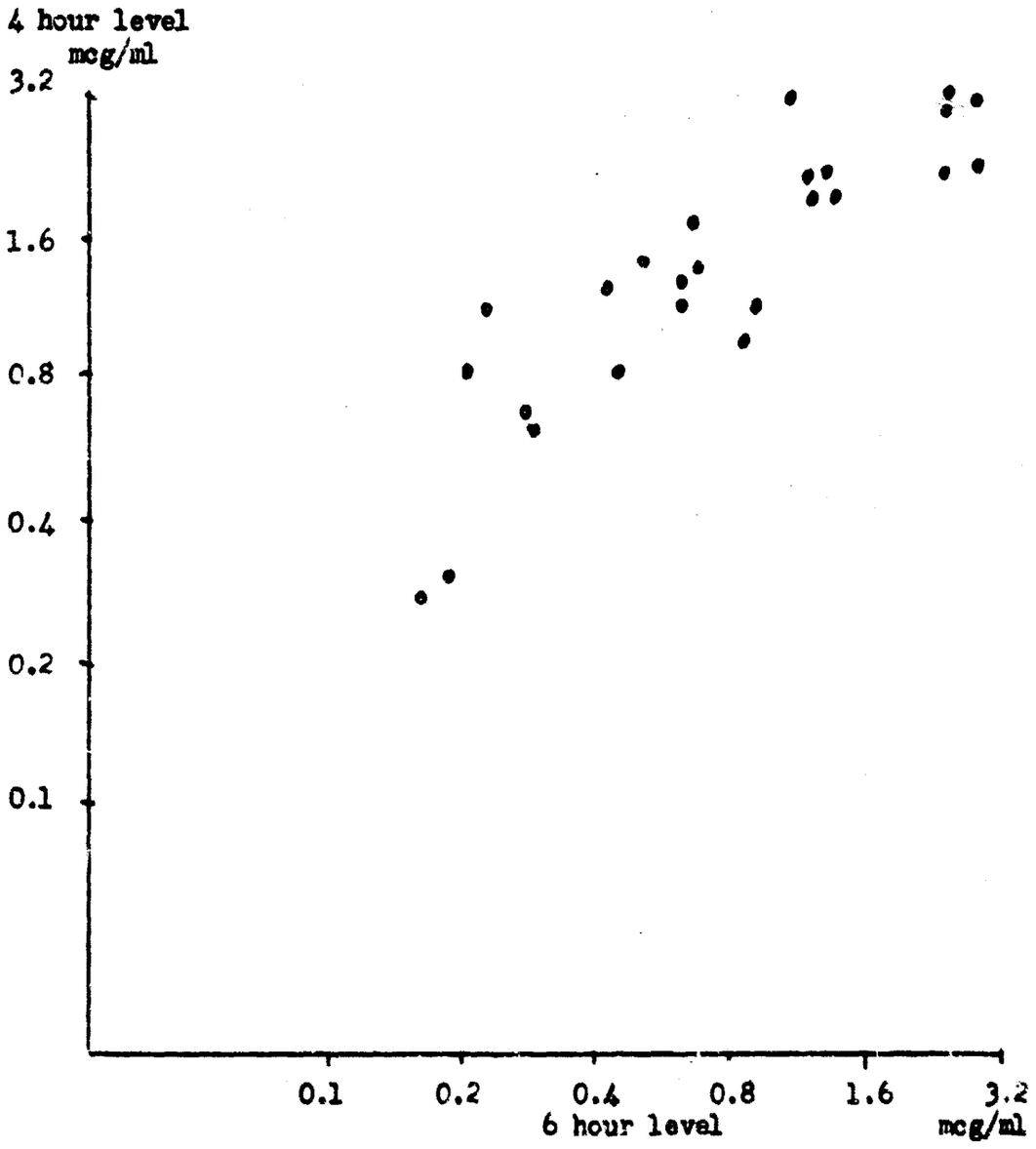


Fig. 3 Relationship between Blood Levels 4 and 6 hours after Administration of 16 mg/kg of INH to Monkies (25 animals from Malaya)

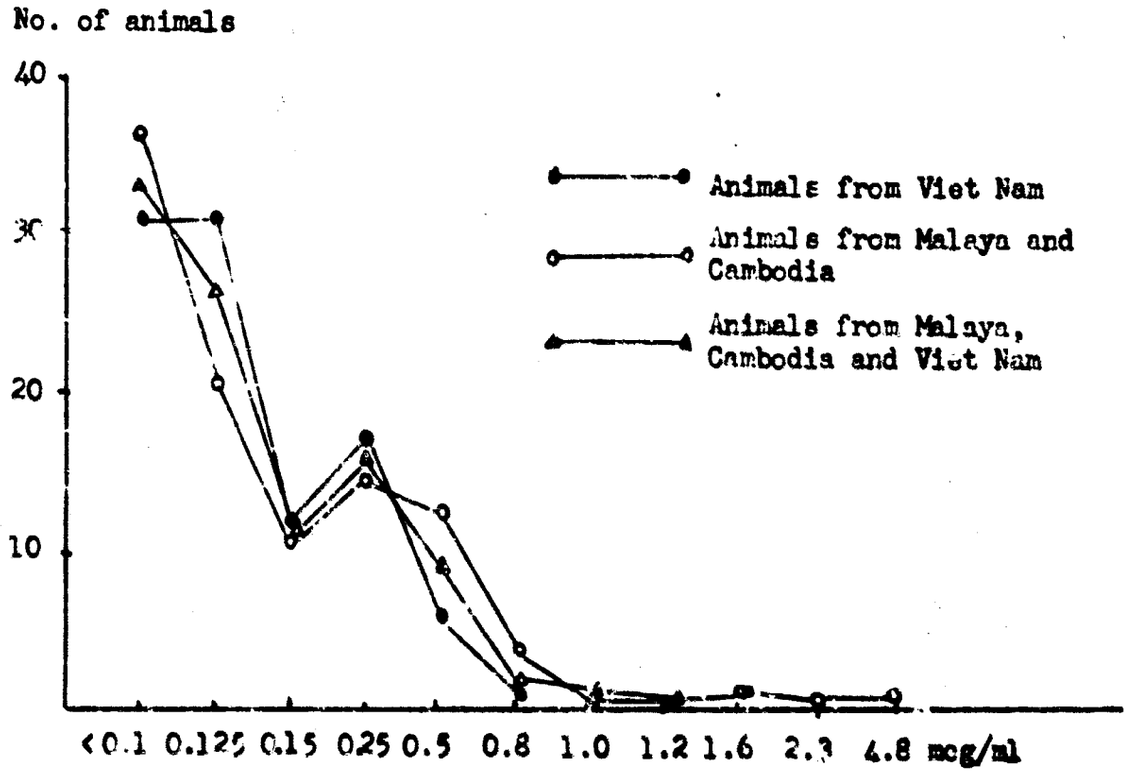


Fig. 4 Distribution Curve of Biologically Active Isoniazid Levels 6 hours after the Dose of 4mg/kg of INH

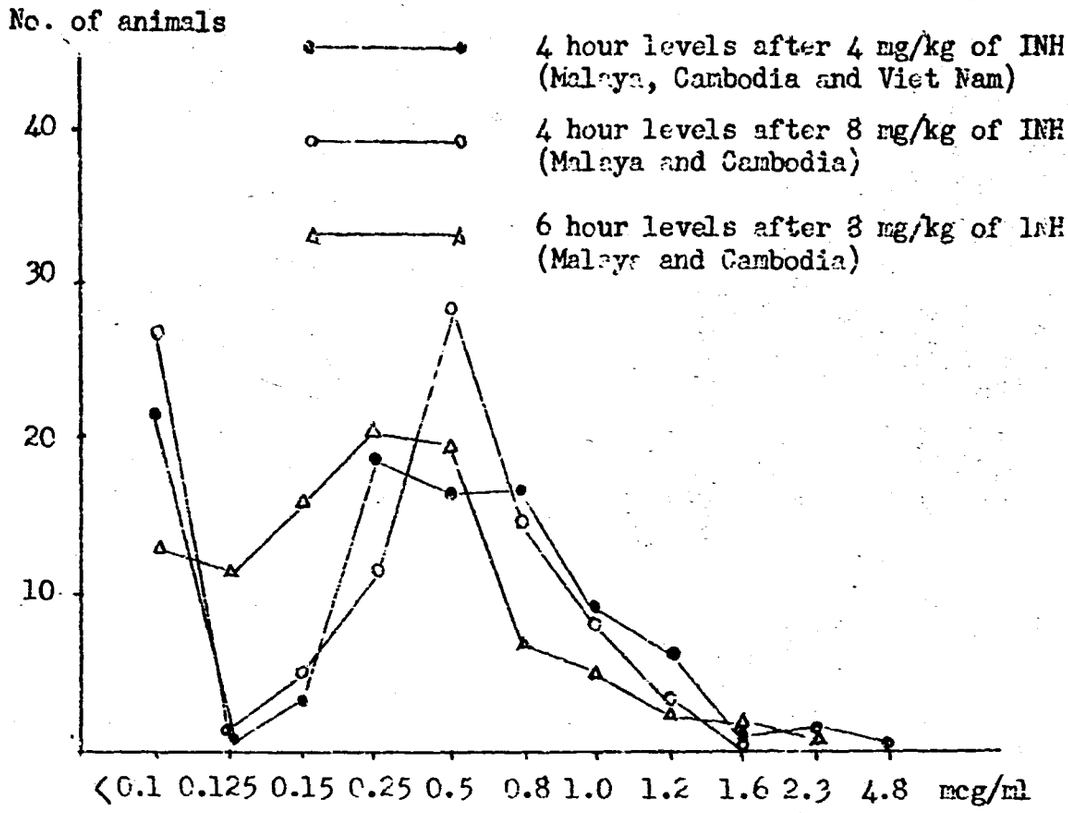


Fig. 5 Frequency Distribution Curve of Biologically Active Isoniazid Levels of Monkies

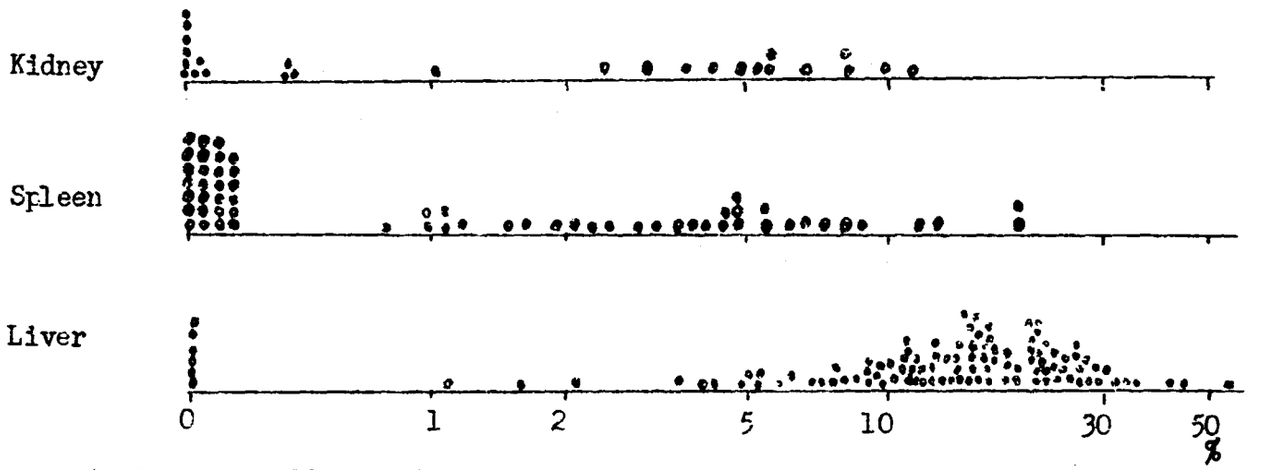


Fig. 6 Frequency Distribution of Rate of Acetylation of Isoniazid in the Liver, Spleen and Kidney

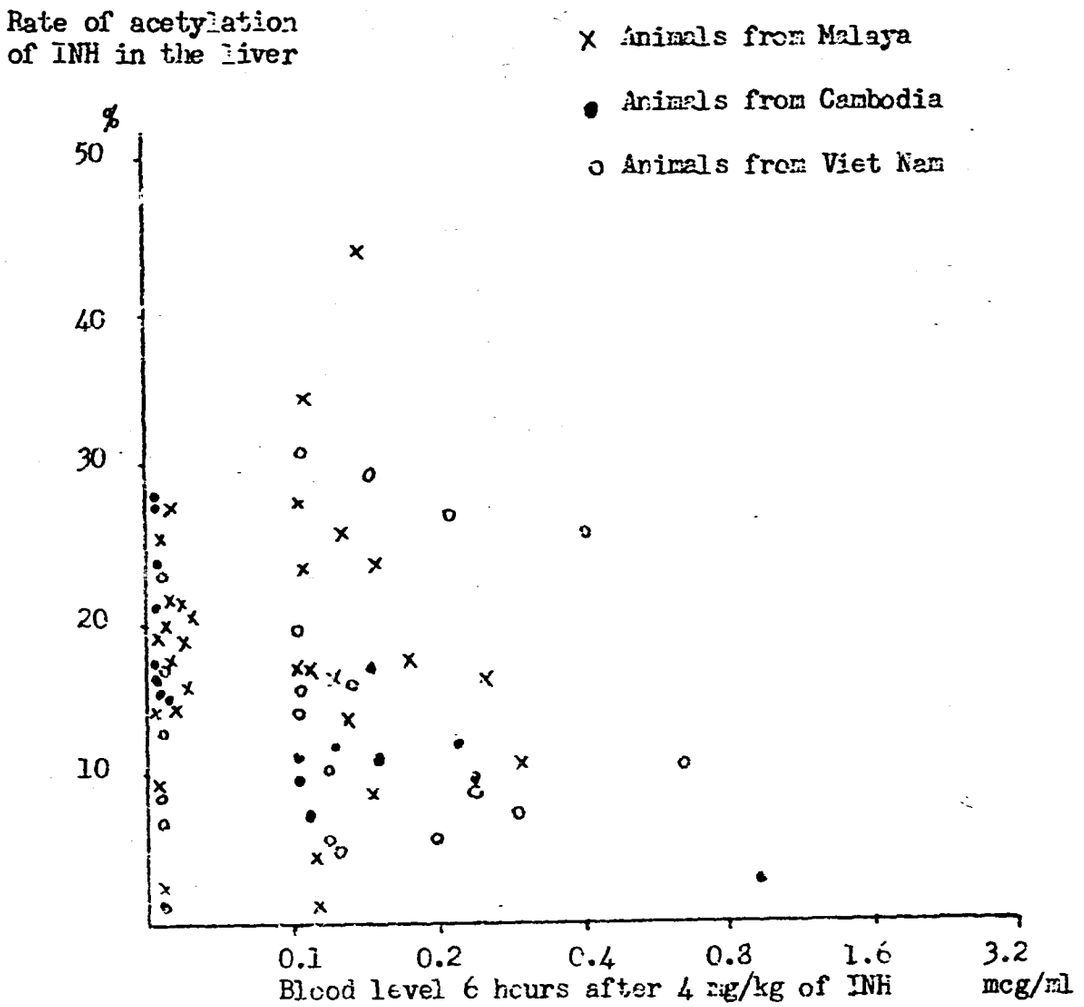


Fig. 7 Relationship between Isoniazid Plasma Level and Rate of Acetylation in Monkey Liver

PART II

Preliminary Report on A New Biochemical Method for Determining Total and Free Isoniazid in Biological Fluid

INTRODUCTION

A number of biochemical methods have been published for determining free isoniazid concentration in serum or urine (Short (10), Hughes (3), Hunter (4), Cuthberton et al. (2), Scott (9), Scarid (8), Poole and Meyer (7), Maher et al. (5), Belles and Littlemar (1), Peters (6), etc.). Being quite difficult to have an accurate value for the concentration below 1 mcg/ml by employing the techniques above mentioned except the Peter's method, we have used the vertical diffusion culture method—a kind of bioassay method—for determining the blood level in our previous reports.

Recently a new biochemical method which enables us to measure a relatively low concentration of free isoniazid has been develped in our laboratory.

PROCEDURE

1. Reagent and Vessel

   a. Fig. 1 Extraction vessel

2. Quantitative Determination of Total INH

   a. Quantitative determination of total INH in urine

3. Quantitative Determination of Free INH

   a. Quantitative determination of free INH in aqueous solution
   b. Quantitative determination of free INH in serum
   c. Quantitative determination of free INH in urine

RESULTS

1. Calibration Curve

   a. Fig. 2 Calibration curve of INH in aqueous solution
   b. Fig. 3 Calibration curve of INH in serum
   c. Fig. 4 Calibration curve of INH in urine

2. Recovery

   a. Table 1 Recovery tests of INH in aqueous solution
   b. Table 2 Recovery tests of INH in aqueous solution, serum & urine
   c. Table 3 Duplication test

3. Comparison between Bioassay and Chemical Assay

   a. Table 4 Comparison of chemical assay and biological assay of INH in aqueous solution
   b. Table 5 Comparison of chemical assay and biological assay of INH in serum
   c. Table 6 Free INH concentration in serum after oral administration of INH (4 mg/kg body weight)

4. Determination of INH Derivatives

   a. Fig. 5 Determination of free INH in aqueous solution of INH and related substances by means of our method
   b. Fig. 6 Recovery of free INH in aqueous solution of different kinds of INH derivatives

COMMENT & SUMMARY

Although it is needless to say that more detailed critical examination is necessary to put it in practical use, our new method for determining total and free INH in biological fluid (tangstate method) seems to be more reliable and convenient than most of biochemical techniques published hitherto. As the color at the end reaction is indigo blue, it can be quite easily distinguished. We are going to investigate in our future study whether this method would be able to replace the bioassay method, especially the vertical diffusion culture method.

PROCEDURE

1. Reagent and Vessal

An extraction vessel with ground stopper as indicated in Fig. 1 was used.

Reagents used were as follows:

1) $(NH_4)_2SO_4$ (anhydrous crystal)
2) 1N-NaOH aq. solution
3) N/10-Hcl aq. solution
4) Dichlorethan
5) Isoamylalcohol
6) 5%-NaCN aq. solution
7) P-tangstate reagent
   (Brown's uric acid reagent,
   Brown, H.J.: J. Biol. Chem.
   158: 601, 1945)
8) 66% urea aq. solution

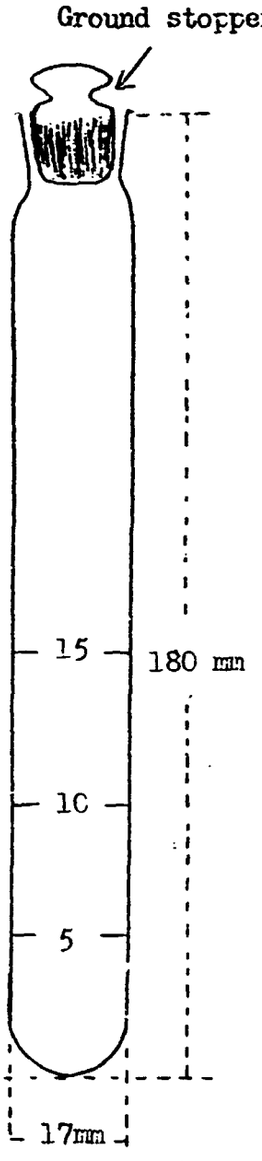
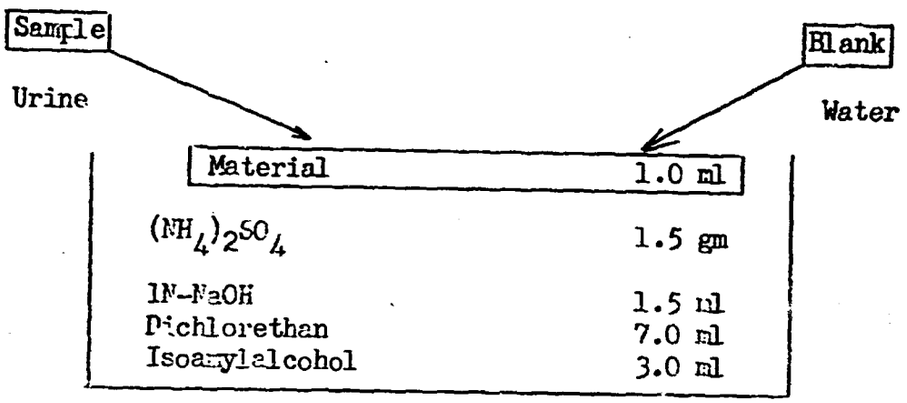


Fig. 1

2. Quantitative Determination of Total INH

Quantitative Determination of Total INH in Urine



Shake for 15 minutes
Centrifugation (1500 r.p.m.) 5 minutes

Upper layer (solvent layer)	5 ml
N/10-HCl	5 ml

Shake for 15 minutes
Centrifugation (1500 r.p.m.) 5 minutes

Upper layer (HCl layer)	3 ml

Place in a boiling water bath for one hour

↓

Cool

↓

Make up to 15 ml with N/10-HCl (X)

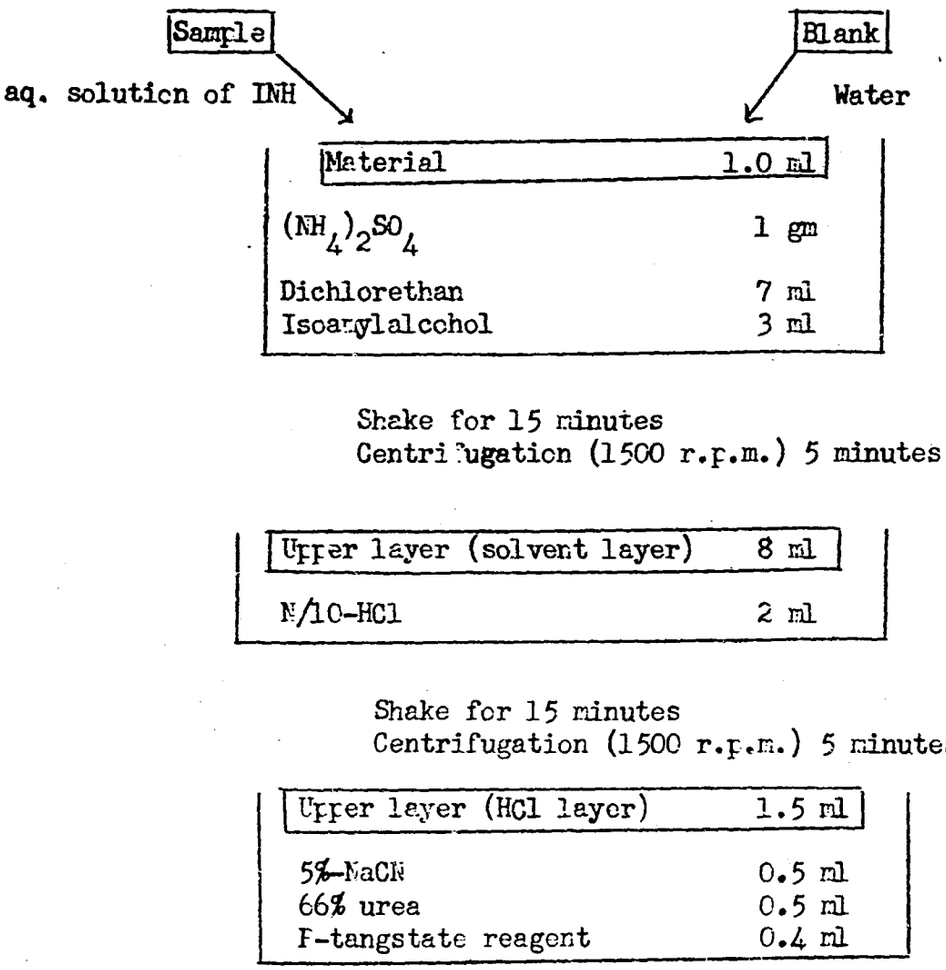
(X)	1.5 ml
5%-NaCN	0.5 ml
66% Urea	0.5 ml
Tangstate reagent	0.4 ml

Place for one hour at room temperature

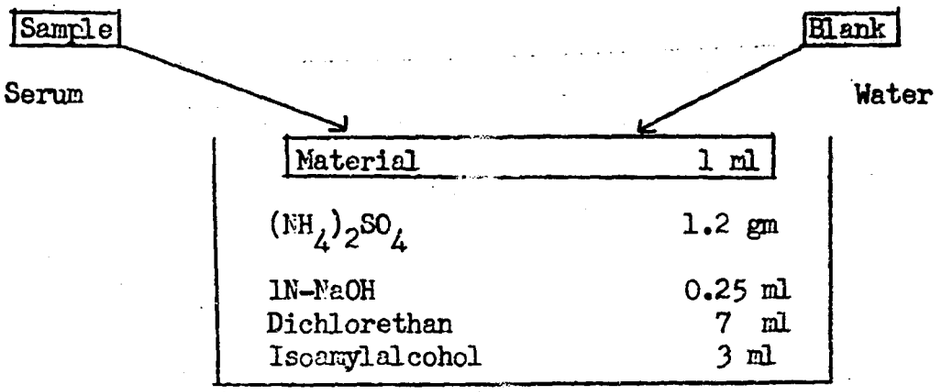
Optical density of the Sample at 660 mμ was read against the Blank.

3. Quantitative Determination of Free INH

Quantitative Determination of Free INH in Aqueous Solution



Quantitative Determination of Free INH in Serum



Shake for 15 minutes
Centrifugation (1500 r p.m.) 5 minutes

Upper layer (Solvent layer)	8 ml
N/10-HCl	2 ml

Shake for 15 minutes
Centrifugation (1500 r.p.m.) 5 minutes

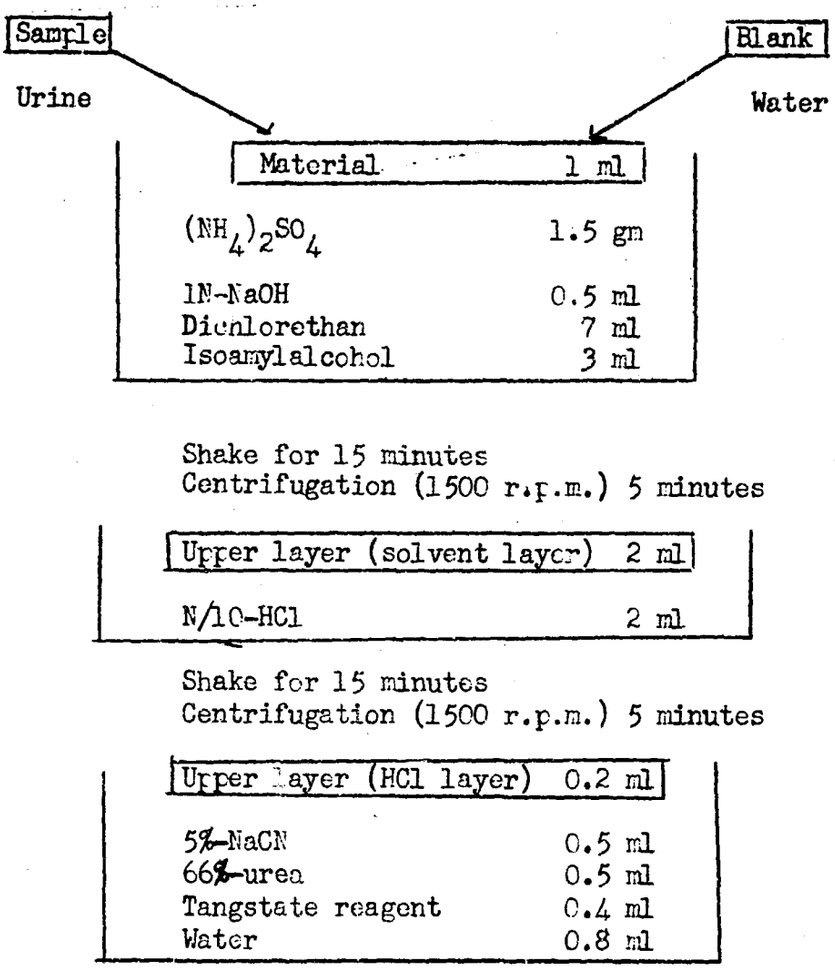
Upper layer (HCl layer)	1.5 ml
5%-NaCN	0.5 ml
66%-urea	0.5 ml
Tangstate reagent	0.4 ml

Place for one hour at room temperature

↓

Optical density of the Sample at 660 mμ was read against the Blank.

Quantitative Determination of Free INH in Urine



Optical density of the Sample at 660 mμ was read against the Blank.

RESULTS

1. Calibration Curve

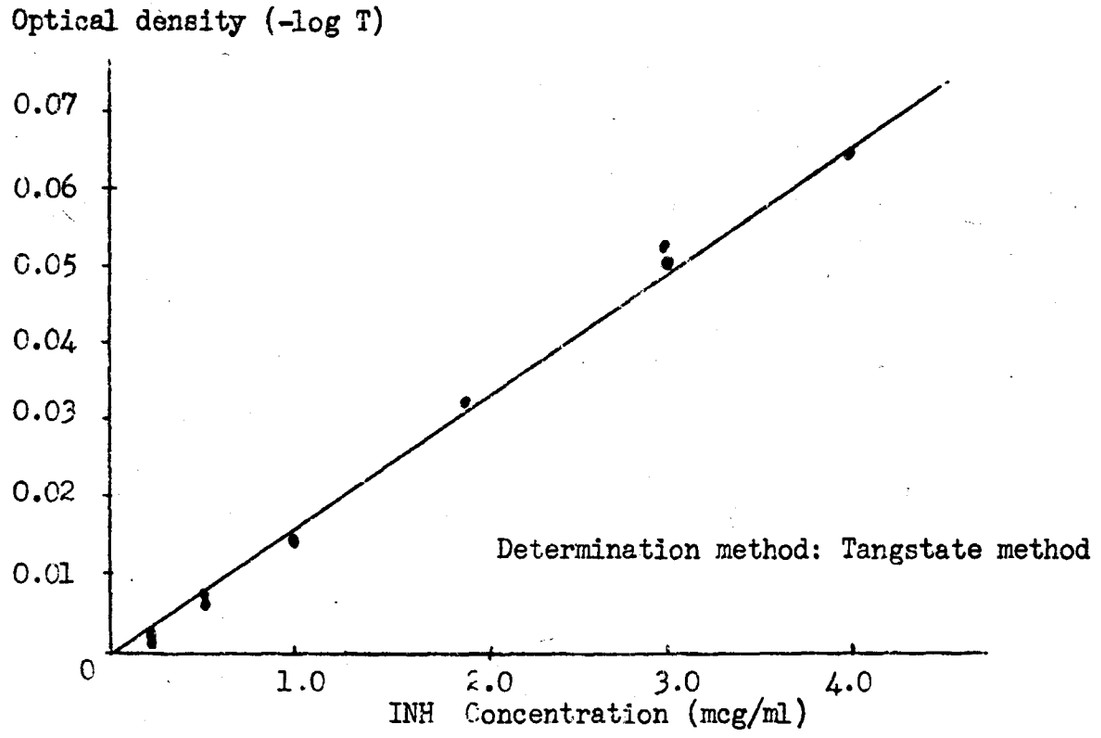


Fig. 2 Calibration Curve of INH in Aqueous Solution

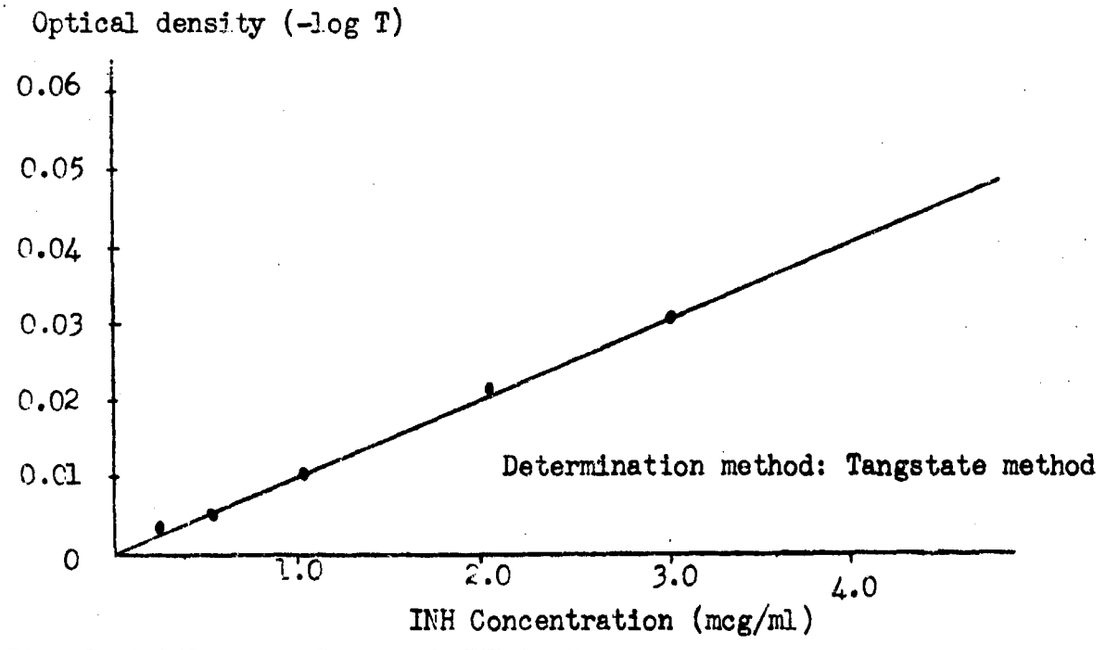


Fig. 3 Calibration Curve of INH in Serum

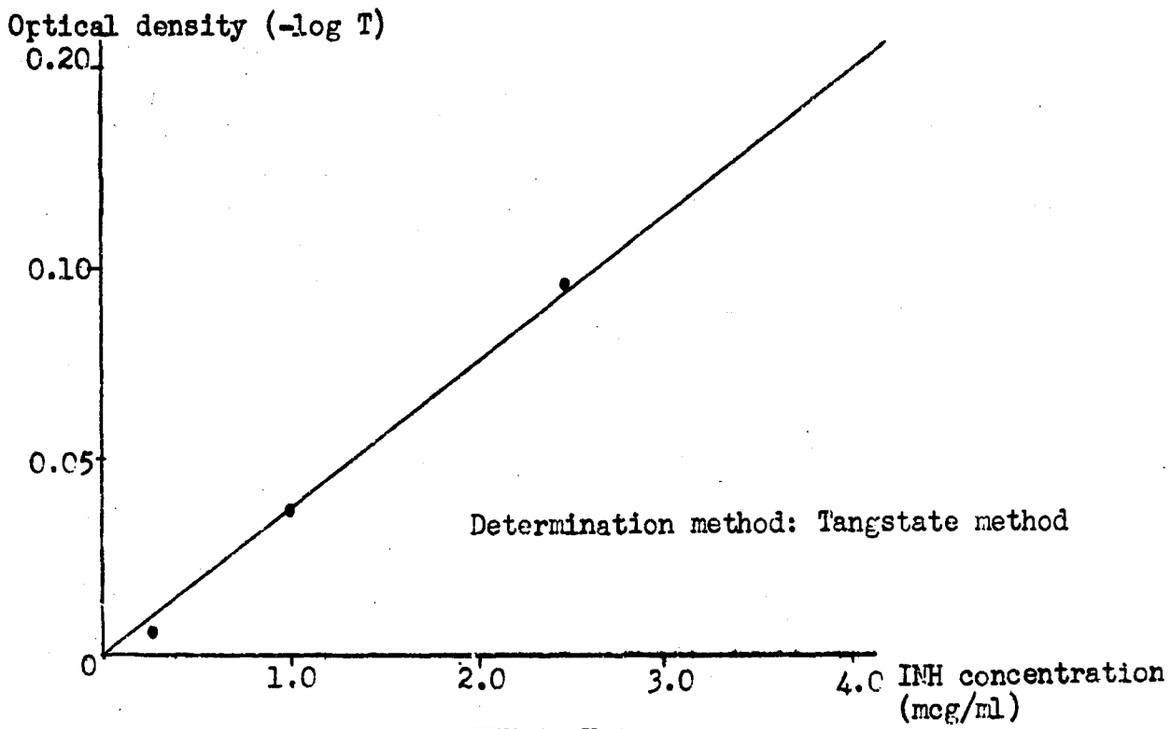


Fig. 4 Calibration Curve of INH in Urine

2. Recovery

Table 1 Recovery Tests of INH in Aqueous Solution

	3 mcg/ml	1 mcg/ml	0.5 mcg/ml
Dupli. No. 1	0.045	0.014	0.010
2	0.050	0.010	0.008
3	0.049	0.019	0.005
4	0.046	0.014	0.008
5	0.054	0.016	0.009
Mean	0.049 ±0.0032	0.0146 ±0.0029	0.008 ±0.0017
Recovered	3.0 mcg/ml	0.9 mcg/ml	0.5 mcg/ml
St. D.	±0.2	±0.18	±0.11

Table 2 Recovery Tests of INH in Aqeous Solution, Serum and Urine

	INH Concentration 3 mcg/ml		
	in aq. solution	in serum	in urine
Dupli.No. 1	0.052	0.030	0.065
2	0.049	0.030	0.066
3	0.051	0.030	0.062
Mean	0.0507	0.030	0.0643
Recovered	3.08 mcg/ml	3.00 mcg/ml	

Table 3 Duplication Test

Dupli. No. 1	0.011	Material: 1 mcg/ml in serum
2	0.009	
3	0.009	
4	0.011	Mean 0.010 ±0.001
5	0.010	
6	0.010	Recovered 1 mcg/ml
7	0.009	St. D. ± 0.1
8	0.010	

3 . Comparison between Bioassay and Chemical Assay

Table 4 Comparison of Chemical Assay and Biological Assay of INH in Aqueous Solution

INH concent. in aq. sol.	Chemical assay mcg/ml	Biological assay mcg/ml
3 mcg/ml	2.75	3.20
"	3.06	2.58
"	3.00	2.58
"	2.81	3.20
"	3.30	2.41
Mean , St. d.	2.98 ±0.20	2.79 ±0.34
1 mcg/ml	0.87	1.09
"	0.63	1.09
"	1.17	1.09
"	0.87	1.31
"	0.98	0.94
Mean , St. d.	0.90 ±0.18	1.11 ±0.12
0.5 mcg/ml	0.63	0.62
"	0.50	0.49
"	0.32	0.49
"	0.63	0.44
"	0.56	0.44
Mean , St. d.	0.53 ±0.13	0.50 ±0.07

Table 5 Comparison of Chemical Assay and Biological Assay of INH in Serum

INH concent. in serum	Chemical assay (a)	Chemical assay (b)	Biological assay (a)
3mg/ml	2.9mcg/ml	3.0mcg/ml	2.58mcg/ml
2 "	1.5	2.1	1.68
1 "	1.0	1.0	0.58
0.5 "	0.9	0.5	0.35
0.2 "	0.1	0.3	0.18

Chemical assay (a) and bioassay (a) were simultaneously determined.

Table 6 INH concentration in serum after administration of INH (4 mg/kg of body weight)

Hour	Chemical assay	Biological assay
1	3.2 mg/ml	>3.2 mcg/ml
2	2.0	>3.2
3	0.9	2.41
4	0.8	1.04
5	0.7	0.65
6	0.5	0.42

4. Determination of INH Derivatives

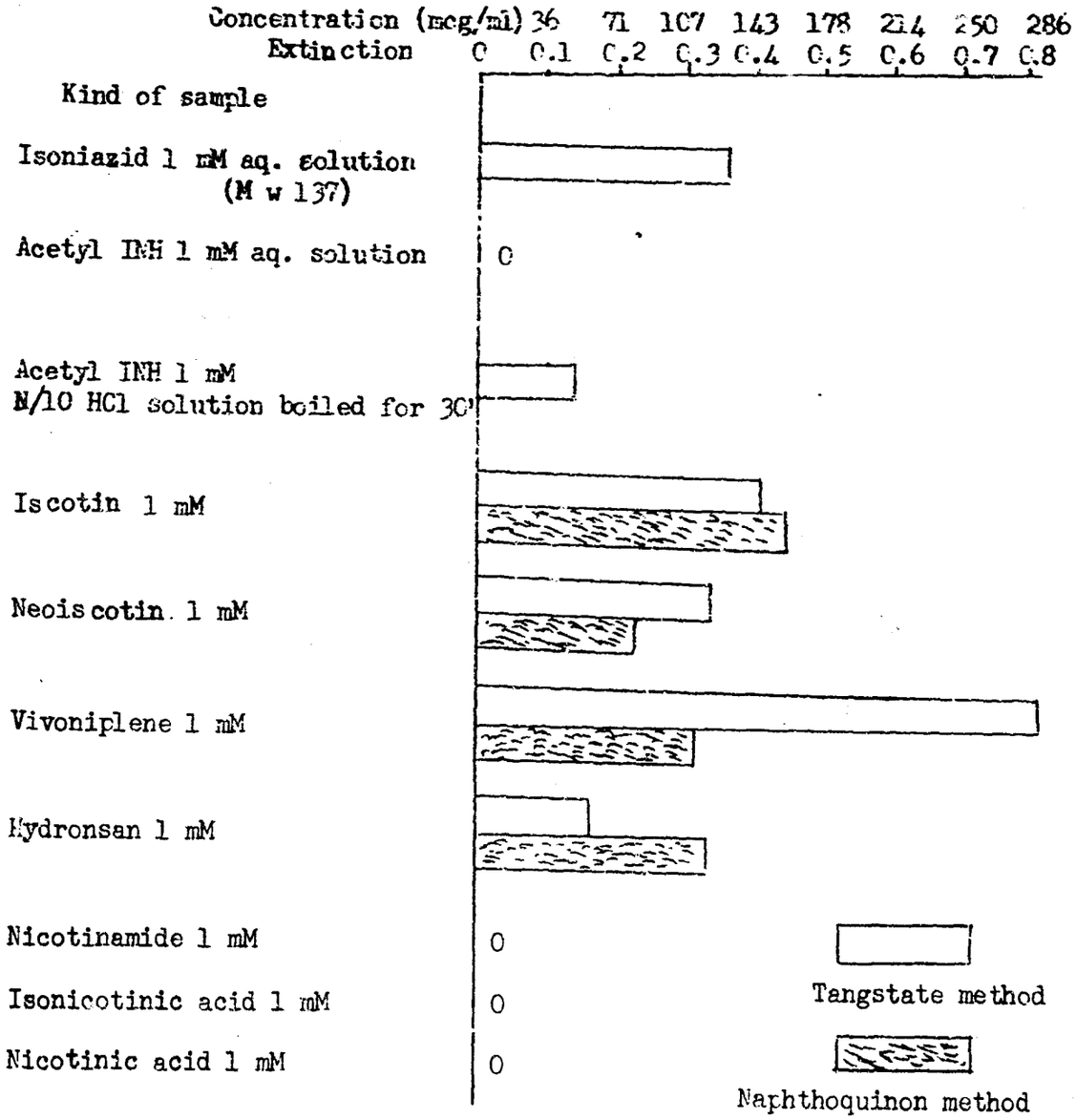


Fig. 5 Determination of Free INH in Aqueous Solution of INH and Related Substances by Means of Our Method

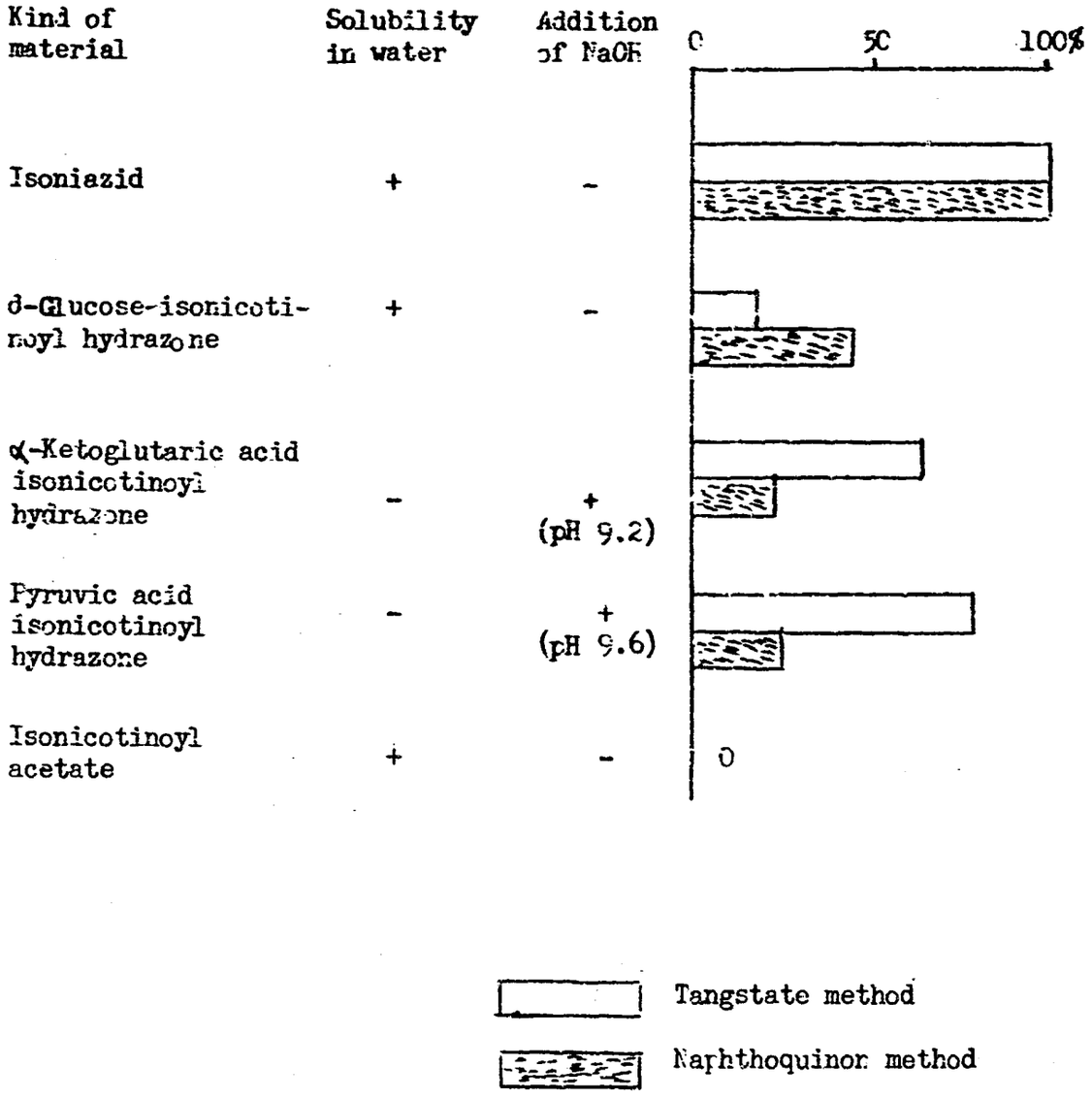


Fig. 6 Recovery of Free INH in Aqueous Solution of Different Kinds of INH Derivatives

REFERENCES

1) Belles, Q.C. & Littleman, M.L.: Am. Rev. Resp. Dis. 81, 364, 1960

2) Cuthberton, W.F.J., Ireland, D.M. & Wolff, W.: Bioch. J. 55, 669,1953

3) Hughes, H.B.: Trans 15th Conf. Chemoth. Tuberc. 574, 1956

4) Hunter, G.: Brit. Med. J. i, 585,1955

5) Maher, J.R., Whitney, J.M., Chambers, J.S. & Stanonis, D.J.: Am. Rev. Tuberc. Pulm. Dis. 76, 852, 1957

6) Peters, J.H.: Am. Rev. Resp. Dis. 81, 485, 1960

7) Poole, N.F. & Meyer, A.E.: Proc. Soc. Exp. Biol. Med. 98, 375, 1958

8) Scardi, V.: Clin. Chim. Acta 2, 134, 1957

9) Scott, E.G.W.: J. Pharm. Pharmacol. 4, 681, 1952

10) a. Short, E.I.: Lancet i, 656, 1954

b. Short, E.I.: Tubercle 42, 218, 1961

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13. ABSTRACT

This paper is concerned with a technical improvement in PERT Critical Path Methodology. The approach utilizes the following devices:

(a) A classification of networks into different types depending on their degree of involvement and complexity.

(b) An operational calculus by which the distribution of critical times will be derived by numerical analysis, notably numerical integration. This method will provide the solution to the problem for the basic types of networks.

(c) A Monte Carlo procedure providing an approximate solution for the more involved networks.

(d) Analytic solutions for particularly simple networks and particularly simple distributions of completion times. These are mainly used for illustration purposes.

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It is highly desirable that the abstract of classified reports be unclassified. Each paragraph of the abstract shall end with an indication of the military security classification of the information in the paragraph, represented as *(TS)*, *(S)*, *(C)*, or *(U)*.

There is no limitation on the length of the abstract. However, the suggested length is from 150 to 225 words.

14. KEY WORDS: Key words are technically meaningful terms or short phrases that characterize a report and may be used as index entries for cataloging the report. Key words must be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical context. The assignment of links, rules, and weights is optional.